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Journal of Chromatography B, 856 (2007) 337–342
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Sensitive determination of trimetazidine in spiked human plasma by HPLC with fluorescence detection after pre-column derivatization with 9-fluorenylmethyl chloroformate

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Received 25 January 2007; accepted 22 June 2007
Available online 6 July 2007

Abstract

A high-performance liquid chromatographic method for the determination of trimetazidine dihydrochloride (TMZ) in spiked human plasma is described. The method is based on the pre-column derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) using the fluorimetric detection technique. Fluoxetine HCl (FLX) was used as internal standard. Both, TMZ and FLX were completely derivatized after heating at 50 °C for 20 min in borate buffer pH 8.0. Samples were analyzed by high performance liquid chromatography (HPLC) using Zorbax-TMS column (250 mm × 4.6 mm, i.d., 5 μm) and mobile phase consist of acetonitrile, methanol and 20 mM sodium acetate pH 4.7 (44:6:50; v/v/v). Fluorescence detector (FLD) was adjusted at excitation and emission wavelengths; 265 and 311 nm, respectively. The linearity of the method was in the range of 4.5–200 ng/ml. Limits of detection (LOD) and quantification (LOQ) were 1.5 and 4.5 ng/ml, respectively. Trimetazidine recovery was 96.5 ± 1.3% ($n = 6$; RSD = 2.1%).

Keywords: Trimetazidine; Fluoxetine; FMOC; Plasma; Chromatography

1. Introduction

Trimetazidine [1-(2,3,4-trimethoxybenzyl)-piperazine dihydrochloride] regulates ionic and extracellular exchanges, corrects ischemia-induced abnormal flow of ions across the cell membrane, and prevents cellular edema caused by anoxia [1–4]. The modified release TMZ tablet is used for the management of long-term angina pectoris [5]. Derivatives of 9-fluorenylmethyl chloroformate (FMOC-Cl) have been widely used for HPLC determination of a large variety of amines [6] but not applied for TMZ or FLX. Courte and Bromet described an HPLC method for the determination of TMZ with fluorescence detection [7]. The method was based on derivatization of TMZ with dansyl chloride. The calibration range of dansylated TMZ was from 10 to 500 ng/ml plasma. Other reported methods for determination of TMZ in biological fluids included gas chromatography–mass spectrometry (GC–MS) [8] and HPLC with UV detection at 240 nm [9]. In addition, TMZ has been determined using spectrophotometric methods based on charge transfer complexes formation using, 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ), 7,7,8,8-tetracyano-quinodimethane (TCNQ), *p*-chloranil, and complexation with iron(III) chloride [10,11]. Other reported methods have been adopted for the analysis of TMZ in bulk or pharmaceutical dosage forms which include voltammetry and HPLC with UV detection [12–15]. With the exception of the HPLC-fluorescence procedure [7], all other analytical methods do not confer enough sensitivity for monitoring low TMZ levels in plasma. The British Pharmacopoeia cited an HPLC method with UV detection at 240 nm for purity testing of TMZ in bulk form [16].

Therefore, the current work was designed to develop a highly sensitive and selective procedure for determining TMZ in plasma using 9-fluorenylmethyl chloroformate as a derivatizing reagent and subsequent measurement using HPLC with fluorescence detection.

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doi:10.1016/j.jchromb.2007.06.036

2. Experimental

2.1. Chemicals and reagents

All solvents were of HPLC grade, Merck, Darmstadt, Germany. All other materials were of analytical grade. 9-Fluorenylmethyl chloroformate (97%) purchased from Sigma–Aldrich, Germany. Trimetazidine dihydrochloride was obtained as a gift from Servier Laboratories, France. Fluoxetine HCl [*N*-methyl-γ-[4-(trifluoromethyl)phenoxy]benzenepropanamine] was obtained as a gift from Eli Lilly Company, Indianapolis, IN, USA. Blank drug-free human plasma was obtained from the internal plasma bank of King Abdulaziz University Hospital, Jeddah, Saudi Arabia.

2.2. Equipment

The HPLC system consisting of an Alliance Waters separations module 2695, waters 2996 Photodiode array detector, and Waters 2475 multi λ fluorescence detector (Milford, MA, USA) was used. Column heater was set to $30 \pm 2\,^{\circ}C$. HPLC system control and data processing was performed by Empower software (Build 1154, Waters). Screw capped V-shaped vials 300-μl, with PTFE liners were used (Alltech, GmbH, Unterhaching, Germany). Heating oven (Heraeus, Kendro, Hanau, Germany) was adjusted at 50 °C. Calibrated digital micro-transfer pipettes 5–250 μl, Brand, Wertheim, Germany was used.

2.3. Chromatographic conditions

Analytes were separated isocratically on a Agilant Zorbax-TMS column (4.6 mm i.d. × 25 cm, 5 μ particle diameter) protected with Agilent Zorbax TMS pre-column (Agilent Technologies, Palo Alto, CA). The mobile phase was a mixture of acetonitrile, methanol and 20 mM sodium acetate buffer pH 4.7 (44/6/50, v/v/v) and the flow rate was 1 ml/min. The fluorescence detector was set at 265 and 311 nm as excitation and emission wavelengths, respectively. The column was washed after each 10 repetitive injections with a mobile system consisting of acetonitrile:methanol:water (40:40:10, v/v/v) for 30 min.

2.4. Derivatization reagent, internal standard solution and buffer solution

Ten milligrams of 9-fluorenylmethyl chloroformate (FMOC-Cl) was accurately weighed into 10-ml volumetric flask, dissolved in about 5 ml acetonitrile, and diluted to the volume with acetonitrile. A volume of 0.50 ml from this solution was further diluted to 10-ml with acetonitrile and 50 μl from final diluted solution was used for derivatization.

Internal standard solution was prepared by dissolving 10 mg of fluoxetine HCl (FLX) in 100 ml water. One milliliter from this solution was further diluted to 100 ml to give a final concentration of 1 μg/ml. A volume of 50 μl from this solution was used as an internal standard.

Borate buffer pH 8.0 ± 0.1 was prepared by mixing 100 ml of a solution containing 1.238 g of boric acid, 1.490 g of potassium chloride and 8.0 ml of 0.2 M sodium hydroxide. This mixture was then diluted to 400 ml with water and the pH was checked by calibrated pH-meter.

2.5. Standard solutions and quality control samples

Trimetazidine dihydrochloride (TMZ) 0.4 mg/ml aqueous solution was prepared and used for calibration and quality control samples preparation. Appropriate dilutions in water were prepared from this stock solution to obtain calibration standards in the range of 90–4000 ng/ml and quality control samples of 4.5, 100 and 200 ng/ml.

Dilutions for calibration standards were prepared daily, while quality control (QC) samples were prepared in plasma, divided in small aliquots and stored at $-20\,^{\circ}C$ until use. A sample volume of 1 ml of QC sample was extracted and analyzed at time intervals of 0, 10 and 30 days using FLX as an internal standard.

2.6. General derivatization procedure

A volume of 50 μl from the TMZ standard solution and 50 μl the FLX solution were transferred to an autosampler glass vial (capacity = 300-μl) using digital micropipette. A volume of 150 μl borate buffer pH 8.0 and 50 μl FMOC-Cl solutions were added. The vial was capped, swirled, and left to stand in hot air oven at 50 °C for 20 min. The vial was then cooled, and a volume of 10 μl was injected for HPLC analysis.

Blank experiments were carried out to identify any underivatized TMZ or FLX by HPLC-FLD by using water instead of sample solution and acetonitrile instead of FLX or FMOC-Cl solution.

2.7. Calibration curves

2.7.1. Standard solutions for calibration curve

Six standard solutions of TMZ were prepared spanning the range 0.09–4.00 μg/ml. A volume of 50 μl from each TMZ concentration with 50 μl of FLX solution were derivatized as described under general derivatization procedure. A volume of 10 μl was injected for HPLC analysis. The percentage of peak area ratio of FMOC-TMZ to FMOC-FLX was plotted versus the TMZ concentration in pg/μl of the final dilution. The calibration curve was constructed using a least-square regression equation for the calculation of the slope, intercept and correlation coefficient. The calibration range was 15–667 ng/ml of the final injected solution.

2.7.2. Calibration curve of plasma extract

Six serial spiked plasma samples were prepared to contain TMZ spanning the range of 4.5–200 ng/ml. A volume of 1 ml from each spiked plasma sample was extracted and derivatized as described in Section 2.8. The calibration curve was produced by linear regression of percentage of peak-area ratios (FMOC-TMZ to FMOC-FLX) against their respective concentrations in picogram per microliter of the final dilution. The calibration curve was constructed using a least-square regression equation for the calculation of the slope, intercept and correlation